Comparison of Different Cell Disruption Methods and Cell Extractant Buffers for Recombinant Bromelain Expressed in E-coli BL21-A1

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Abtsract

The often-encountered problem such as protein degradation has driven various methods of cell lysis in obtaining recombinant protein post fermentation. In this paper, we compare methods such as homogenization, sonication, sonication with lysozyme and chemical lysis using B-PER reagent with lysozyme to extract the recombinant bromelain from *E. coli* BL21-AI. The sonication process is found to be the most effective in releasing recombinant bromelain without any pre-treatment. To obtain the high quality of protein from sonication method, the influence of different extractant buffer was investigated including phosphate buffer saline (PBS), PBS containing cysteine and EDTA (PBS-CE), and sodium phosphate buffer containing cysteine and EDTA (EB-CE). The highest specific enzyme activity was obtained when it was extracted with EB-CE buffer. Under sodium dodecyl sulfate polyacrylamide gel electrophoresis, the recombinant bromelain showed protein band at 55kDa. In conclusion, the sonication method with extractant buffer containing 100mM phosphate buffer pH7.0 with 15 mM cysteine and 2 mM EDTA (EB-CE) was shown to give high specific activity of recombinant bromelain.

Keywords: Recombinant bromelain, cell disruption, sonication buffer